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(19) (CA) **CANADIAN PATENT** (12)

(54) DI-ENZYMATIC DENTIFRICE

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4 DI-ENZYMATIC DENTIFRICE
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8 ABSTRACT OF THE DISCLOSURE
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11 A di-enzymatic dentifrice is provided which contains
12 an oxidizable substrate and an oxidoreductase enzyme specific
13 to such substrate for producing hydrogen peroxide upon oral
14 application of the dentifrice and further contains a
15 thiocyanate salt and lactoperoxidase for interacting with
16 hydrogen peroxide to produce a hypothiocyanate bacterial
17 inhibitor. An illustrative enzymatic system for this purpose
18 contains glucose, glucose oxidase, potassium thiocyanate and
19 lactoperoxidase.
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2 BACKGROUND OF THE INVENTION
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4 This invention relates to dentifrice compositions and,
5 more particularly, to antiseptic dentifrice compositions
6 wherein hypothiocyanate, a bacterial inhibitor, is produced
7 in situ during oral application of the dentifrice.

8 Dentifrices, in powder, paste, cream and liquid forms,
9 are used for both cosmetic and therapeutic purposes. Consistent
10 with these purposes, dentifrices are formulated to contain
11 active ingredients such as cleansing and polishing materials,
12 as well as various antibacterial and anticaries agents for
13 use as aids in the prevention of tooth decay.

14 It is generally understood in the dental art that
15 certain kinds of tooth decay are initiated by acid etching of
16 the tooth enamel with the source of the acid being a metabolite
17 resulting from bacterial and enzymatic action on food particles
18 in the oral cavity. It is generally accepted that plaque--which
19 is a soft accumulation on the tooth surfaces consisting of an
20 organized structure of microorganisms, proteinaceous and
21 carbohydrate substances, epithelial cells, and food debris--is
22 a contributory factor in the development of various pathological
23 conditions of the teeth and soft tissue of the oral cavity. It
24 has been suggested that the saccharolytic organisms of the oral
25 cavity, which are associates with the plaque cause decalcifi-
26 cation beneath the plaque matrix through metabolic activity
27 which results in the accumulation and localized concentration
28 of organic acids. The etching and decalcification of the enamel

1 may continue until the pulp chamber of the tooth is reached.

2 A wide variety of materials have been considered for
3 use as decay-preventative agents in dentifrice compositions.
4 Some of the substances which have been so considered include
5 para-aminobenzoic acid, a combination of urea and urease to
6 produce ammonia during oral application of the dentifrice,
7 chlorophyll, perflourinated long chain organic compounds,
8 complex iodine, penicillin, benzohydroxamic acid, and glucose
9 oxidase to produce hydrogen peroxide during oral application
10 of the dentifrice.

11 U. S. Patent 2,526,614 (Butterfield, 1950) discloses
12 the incorporation into a dentifrice of an enzyme system
13 comprising urea and urease which produces ammonia in the
14 presence of moisture that is encountered during oral application
15 of the dentifrice. The patentee reports that the action of the
16 ammonia together with residual urea is bacterocidal to
17 acidogenic organisms and antienzymatic to the production of
18 lactic acid by such organisms. In addition, it is pointed out
19 that the action of ammonia produced from this enzyme system
20 dissolves mucin plaques.

21 U. S. Patent 3,427,380 (Kirkland, 1969) discloses
22 that oral organisms produce a capsular material which is a
23 factor in holding plaque together and allowing its further
24 growth and that the oral application of a dentifrice containing
25 para-aminobenzoic acid inhibits capsule formation by such
26 organisms and thereby retards the development of dental plaque
27 without inhibiting the growth of these organisms.

28 U. S. Patent 3,137,634 (Schiraldi, 1964) discloses

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1 that the oral application of a dentifrice composition containing,
2 for example, potassium copper chlorophyllin, dicalcium
3 phosphate dihydrate, and tetrasodium pyrophosphate is useful
4 in the treatment of gum diseases such as periodontal disorders
5 like gingivitis, pyorrhea and trench mouth and, in addition,
6 reduces undesirable breath odors.

7 U. S. Patent 3,227,618 (Dunellen, 1966) in the back-
8 ground portion of the specification, recites that it has been
9 disclosed that treatment of tooth enamel with a mixture of
10 stannous fluoride, hydrogen peroxide and insoluble sodium
11 metaphosphate increases the enamel hardness as described in
12 The Journal of the American Dental Association, May, 1950,
13 Vol. 40, pg. 513-519.

14 Merck Index, 9th Edition, 1976, at page 633, discloses
15 that hydrogen peroxide solution 3% contains 2.5-3.5 wt.% of
16 hydrogen peroxide which is equivalent to 8-12 volumes of oxygen,
17 and that this solution is a topical anti-infective which is
18 useful in pharmaceutical preparations such as mouthwashes,
19 dentifrices, and sanitary lotions.

20 U. S. Patent 4,150,113, (Hoogendoorn et al, 1979) dis-
21 closes an enzymatic dentifrice containing glucose oxidase
22 which acts on glucose present in saliva and tooth plaque to
23 produce hydrogen peroxide. The patentees, after noting that oral
24 bacteria effect glycolysis of food products containing sugars
25 through bacterial enzyme systems having SH-groups, point out
26 that lactoperoxidase, which is present in saliva, provides the
27 means for transferring oxygen from hydrogen peroxide to the oral
28 bacteria resulting in the oxidation of the SH-containing enzymes

1 into inactive disulfide enzymes. It is further disclosed that
2 the dentifrice may be formulated with potassium thiocyanate.

3 U. S. Patent 4,269,822 (Pellico et al, 1981) discloses
4 an antiseptic dentifrice containing an oxidizable amino acid
5 substrate and an oxidoreductase enzyme specific to such substrate
6 for producing hydrogen peroxide and ammonia upon oral application
7 of the dentifrice, with pre-application stability being main-
8 tained by limiting the quantity of any water present in the
9 dentifrice.

10 Morrison et al, Biology of the Mouth, American Associa-
11 tion for the Advancement of Science, 1968, pp. 89-110 disclose
12 that lactoperoxidase, sodium thiocyanate and hydrogen peroxide
13 define an enhanced bacterial inhibitory system.

14 Hoogendoorn et al, Caries Research, 11:77-84, 1977,
15 disclose that the hypothiocyanate ion is the bacterial inhibitor
16 formed by the system containing lactoperoxidase, thiocyanate and
17 hydrogen peroxide.

18 Thomas et al, Journal of Dental Research 60(4), pp.
19 785-796, April, 1981, disclose that the yield or accumulation
20 of hypothiocyanate from the antimicrobial system containing
21 lactoperoxidase, thiocyanate and hydrogen peroxide can be
22 increased by the presence of aminohexoses, namely, glucosamine
23 and N-acetyl glucosamine.

24 The effectiveness of a glucose oxidase dentifrice
25 (U.S. Patent 4,150,133) as a bacterial inhibitor through the
26 production of hypothiocyanate is dependent, to a significant
27 extent, upon the subsisting oral concentration of glucose,
28 potassium thiocyanate and lactoperoxidase as well as hydrogen.

1 peroxide at the time of oral application. The concentration of
2 those ingredients supplied by saliva, including potassium
3 thiocyanate and lactoperoxidase, varies as a direct function of
4 physiological production and salivary flow. Thus, when
5 salivary flow is at a diminished level either as a natural
6 event or as a event arising out of certain types of medical
7 treatment, the oral concentration of potassium thiocyanate and
8 lactoperoxidase will be correspondingly reduced which, in turn,
9 is a limiting factor in the oral production of hypothiocyanate
10 bacterial inhibitor. Accordingly, it would be advantageous to
11 provide a substantially self-contained, hypothiocyanate genera-
12 ting, engymatic dentifrice which is not dependent upon the
13 naturally occurring, oral concentration of glucose, potassium
14 thiocyanate or lactoperoxidase for antibacterial effectiveness.
15 upon oral application of the dentifrice.

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1 SUMMARY OF THE INVENTION

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3 In accordance with the invention, there is provided
4 a di-enzymatic dentifrice containing from about 0.015 to about
5 0.6 millimole of oxidizable substrate and from about 0.5 to
6 about 500 International Units of an oxidoreductase enzyme
7 specific to such substrate for producing hydrogen peroxide upon
8 oral application of said dentifrice and further containing
9 from about 0.0001 to about 0.01 millimole of a
10 thiocyanate salt and from about 0.05 to about 20 International
11 Units of lactoperoxidase for interacting with hydrogen peroxide
12 to produce a hypothiocyanate bacterial inhibitor, wherein each
13 of the aforesaid quantities is based upon one gram
14 of dentifrice; and limiting any water present to an amount
15 not more than about 10 wt. % based on the dentifrice weight to
16 stabilize the dentifrice against the production of hydrogen
17 peroxide prior to oral application of the dentifrice.
18

19 DETAILED DESCRIPTION

20
21 The di-enzymatic dentifrice of this invention comprises
22 a first enzyme system containing an oxidizable substrate and an
23 oxidoreductase enzyme specific to such substrate for producing
24 hydrogen peroxide upon oral application of the dentifrice, with
25 the chemical environment of the oral cavity providing the source
26 of the additional reactant (oxygen) or reactants (oxygen, water)
27 to effect the enzymatic reaction.

28 The components of the first enzyme system which can

1 be incorporated into dentifrice compositions to produce hydrogen
 2 peroxide upon oral application of the dentifrice are illustrated
 3 by the substrate/enzyme combinations set forth in Table I.

4 TABLE I

Oxidizable 5 <u>Substrate</u>	Oxidoreductase 6 <u>Enzyme</u>
(a) B-D-glucose	glucose oxidase
(b) D-galactose	galactose oxidase
(c) Urate	urate oxidase
(d) Choline	choline oxidase
(e) D-amino acids	D-amino acid oxidase
(f) D-glutamate	D-glutamate oxidase
(g) Glycine	glycine oxidase
(h) Glycollate	glycollate oxidase
(i) L-sorbose	L-sorbose oxidase
(j) Primary alcohol	alcohol oxidase
(k) Primary amine	amine oxidase

18 The reactions of representative enzyme systems from
 19 Table I, which are activated in the chemical environment of the
 20 oral cavity to produce hydrogen peroxide, are set forth in
 21 Table II.

22 TABLE II

- 23 (a) Glucose oxidase catalyzes the interaction of Beta-D-glucose,
 water and oxygen to produce hydrogen peroxide and gluconic acid;
- 24 (b) Galactose oxidase catalyzes the interaction of D-galactose
 and oxygen to produce hydrogen peroxide and D-galacto-hexo-
 dialdose;
- 25 (c) Urate oxidase catalyzes the interaction of urate, water

1 and oxygen to produce hydrogen peroxide, allantoin and carbon
2 dioxide;

3 (d) Choline oxidase catalyzes the interaction of choline and
4 oxygen to produce hydrogen peroxide and betaine aldehyde;

5 (e) D-amino acid oxidase catalyzes the interaction of D-amino
6 acids such as the D isomers of proline, methionine, isoleucine,
7 alanine, valine and phenylalanine together with water and
8 oxygen to produce hydrogen peroxide, ammonia and the corres-
9 ponding alpha-keto acids;

10 (f) D-glutamate oxidase catalyzes the interaction of D-glutamate,
11 water and oxygen to produce hydrogen peroxide, ammonia and
12 2-oxoglutarate; and

13 (g) Glycine oxidase catalyzes the interaction of glycine,
14 water and oxygen to produce hydrogen peroxide, ammonia and
15 glyoxylic acid.

16 The characteristics of representative oxidoreductase
17 enzymes identified in Table I, from specific sources, are set
18 forth in Table III.

19 TABLE III

20 (a) Glucose oxidase from A. niger:

21 (i) Molecular weight; 150,000 (Pazur et al., 1965); 153,000
22 (Swoboda, 1969).

23 (ii) Composition: a glycoprotein containing two molecules of
24 flavine-adenine dinucleotide (see: The Merck Index, 9th Ed.,
25 1976, page 532, section 4007 and page 576, section 4291). The
26 amino acid composition has been determined (Pazur et al., 1965).

27 (iii) Isoelectric point: pH 4.2.

28 (iv) Optimum pH: 5.5 with a broad pH range from 4 through 7.

1 (v) Inhibitors: monovalent silver and divalent mercury and
2 copper ions.

3 (b) Galactose oxidase from Dactylium Dendroides:

4 (i) Molecular Weight: 42,000 (Kelly-Falcoz, 1965)

5 (ii) Composition: metalloenzyme containing 1 gram atom of copper
6 per mole (Amaral et al., 1963). The amino acid composition has
7 been determined (Kelly-Falcoz, 1965).

8 (iii) Optimum pH: 7 (Cooper et al., 1959).

9 (c) Urate oxidase (uricase) from hog liver or beef liver:

10 (i) Molecular Weight: 100,000 (Mahler et al., 1955).

11 (ii) Composition: metalloenzyme containing 1 gram atom of
12 copper per mole (Mahler, 1955).

13 (iii) Isoelectric point: pH 6.3.

14 (iv) Optimum pH: 9.

15 (e) D-Amino Acid Oxidase from Hog Kidney:

16 (i) Molecular Weight: 90,000 (Antonini et al., 1966).

17 (ii) Composition: A glycoprotein containing two molecules of
18 flavine-adenine dinucleotide.

19 (iii) Optimum pH: 9.

20 (iv) Inhibitors: certain heavy metals.

21 The oxidizable substrate is generally present in the
22 dentifrice in an amount from about 0.015 to about 0.6 millimole
23 per gram of dentifrice and, preferably, from about 0.025 to about
24 0.1 millimole per gram of dentifrice while the oxidoreductase
25 enzyme specific to the substrate is generally present in the
26 dentifrice in an amount from about 0.5 to about 500 International
27 Units (hereinafter sometimes abbreviated IU) per gram of
28 dentifrice and preferably, from about 10 to about 40 IU per gram

1 of dentifrice. The term millimole identifies that quantity
2 in grams corresponding to the molecular weight of the composi-
3 tion divided by one thousand. The term International Unit(s)
4 identifies that amount of enzyme that will effect catalysis of
5 1.0 micromole of substrate per minute at pH 7.0 and 25° C.
6 Oxidoreductase enzymes are supplied in dry or liquid form with
7 the label specifying the concentration in International Units
8 on a per gram or per milliliter basis, as appropriate.

9 In addition to the first enzyme system comprising
10 oxidizable substrate and oxidoreductase enzyme specific to
11 such substrate for producing hydrogen peroxide, the di-enzymatic
12 dentifrice of this invention is provided with a second enzyme
13 system containing a thiocyanate salt and lactoperoxidase for
14 interacting with hydrogen peroxide to produce a bacterial
15 inhibitor in the form of a negative, monovalent hypothiocyanate
16 ion (OSCN) which exists in solution in an equilibrium with its
17 corresponding salt such as potassium hypothiocyanate (KOSCN).

18 The thiocyanate salts which can be used in the
19 dentifrice include sodium thiocyanate, potassium thiocyanate,
20 ammonium thiocyanate, ferric thiocyanate, cuprous thiocyanate
21 and mixtures thereof. The thiocyanate salt is generally present
22 in the dentifrice in an amount from about 0.0001 to about 0.01
23 millimole per gram of dentifrice and, preferably, from about
24 0.001 to about 0.006 millimole per gram of dentifrice.

25 Lactoperoxidase is a glycoprotein which, in one
26 commercial embodiment, is a lyophilized powder derived from milk.
27 This commercial peroxidase has an activity of 80 IU/mg and a
28 projected molecular weight of 93,000 for L-Tyrosine Iodination.

1 The physical-chemical properties reported for lactoperoxidase
2 include: molecular weight 78,000; partial specific volume
3 0.74; and heme/mole 1.0. Lactoperoxidase is generally present
4 in the dentifrice in an amount from about 0.05 to about 20 IU
per gram of dentifrice and, preferably, in an amount from
5 about 0.1 to about 1.0 IU per gram of dentifrice.
6

7 The di-enzymatic dentrifice of this invention may
8 advantageously be formulated with an aminohexose as, for
9 example, an aminoglucose such as glucosamine, N-acetyl glucosamine
10 or mixtures thereof in order to increase the yield or accumula-
11 tion of the hypothiocyanate ion. The aminoglucose is generally
12 present in the dentifrice in an amount from about 0.001 to about
13 0.002 millimole per gram of dentifrice and, preferably, in an
14 amount from about 0.003 to about 0.001 millimole per gram of
15 dentifrice.

16 Since water promotes the oxidation/reduction reactions
17 of this invention and is also a reactant in certain reactions, the
18 use of water in formulating the dentifrice compositions should
19 be at a relatively low concentration level in order to impart
20 maximum stability and shelf life to the compositions. For this
21 purpose, it has been found to be essential to limit any water
22 present in the dentifrice to an amount not more than about 10 wt.
23 %. In view of this water limitation, a non-aqueous fluid carrier
24 is advantageously employed in the toothpaste formulation so as
25 to provide the formulation with pressure responsive flow
26 characteristics. Any suitable non-aqueous fluid may be used
27 for this purpose. Organic fluid carriers, such as glycerine or
28 propylene glycol provide a stable toothpaste environment for

1 for the enzyme systems of this invention. The non-aqueous fluid
2 carrier is generally present in the dentifrice composition in
3 an amount from about 30 to about 60 wt.% and, preferably, in
4 an amount from about 45 to about 55 wt.%.

5 Where the products of the activated enzyme system
6 include a weak organic acid, it is advantageous to formulate the
7 dentifrice with a buffering agent to neutralize the organic acid.
8 A suitable buffering agent is sodium bicarbonate which can be
9 present in the dentifrice in an amount up to about 6 wt.% as,
10 for example, in an amount from about 4 to about 6 wt.%.

11 Dentifrices, especially toothpaste, are preferred oral
12 compositions for the purpose of this invention. Dentifrice com-
13 positions typically contain an abrasive polishing material
14 and a surfactant as well as flavoring, sweetening and coloring
15 agents. Toothpaste usually also contains humectants and thick-
16 eners.

17 Any abrasive polishing material which does not
18 excessively abrade dentin and is compatible with the oxidore-
19 ductase enzymes described herein can be used in the compositions
20 of this invention. These include, for example, calcium carbonate,
21 calcium pyrophosphate, dicalcium phosphate, zirconium oxide
22 and aluminum oxide. The abrasive polishing material is usually
23 present in toothpaste in an amount from about 20 to 60 wt.%.

24 The surfactants which can be used are those which
25 yield substantial levels of foam and which are otherwise accept-
26 able for use in the oral cavity and compatable with the oxidore-
27 ductase enzymes. A suitable surfactant is sodium lauryl sulfate.
28 However, a protein surfactant or dioctyl sodium sulfosuccinate

1 surfactant is preferred because these surface active materials
2 have been found to be more compatible with the oxidoreductase
3 enzymes. The surfactants can be employed at concentration levels
4 ranging from about 0.5 to about 5.0 wt.-%.

5 The di-enzymatic dentifrice, in the form of a
6 toothpaste, can be prepared in any suitable manner as, for
7 example, by blending the dry ingredients into the liquid
8 ingredients, with agitation, until a smooth mixture is obtained.
9 The addition of any surfactant to the mixture should take place
10 as the last step in order to minimize foaming of the batch.

11 EXAMPLES

12 The following examples further illustrate the composi-
13 tions of this invention. The term "Maypon" used in the examples
14 is the trademark of Stepan Chemical Company, Fieldsboro, N.J.,
15 for a potassium coco condensate of hydrolyzed animal protein
16 having a molecular weight between 750 and 1,500 and supplied
17 as an aqueous solution containing 34 to 40% solids. The term
18 "Super-Pro" used in the examples is the trademark of Stepan
19 Chemical Company for an aqueous solution of sorbitol and
20 triethanolamine condensate of hydrolyzed animal protein having
21 a molecular weight between 750 and 1,500 with the solution having
22 a solids content from 62-70%. The term "DSS" used in the
23 examples is the abbreviation for dioctyl sodium sulfosuccinate.
24 Distilled water is employed in the examples.

25 The term "Silcron G-910" used in the examples is the
26 trademark of SCM/Glidden for a polishing agent comprising a
27 micron-sized hydrated silica gel.

1 EXAMPLE 1

2 This example compares the antibacterial properties
 3 of a di-enzymatic toothpaste of this invention containing glucose,
 4 glucose oxidase, potassium thiocyanate and lactoperoxidase with
 5 the antibacterial properties of an enzymatic toothpaste con-
 6 taining glucose oxidase alone as taught in U.S. Patent 4,150,113
 7 (Hoogendorn, 1979).

8 Enzymatic toothpastes were prepared having the
 9 following formulations:

10		<u>Weight, grams</u>	
11	<u>Composition</u>	<u>1A</u>	<u>1B</u>
12	Glycerine (99%)	48	48
13	Propylene glycol	5	5
14	Sodium bicarbonate	1.9	1.9
15	Silcron G-910	35	35
16	Water	2	2
17	DSS	2	2
18	Glucose oxidase (100,000 IU/g)	0.125g (12,500IU)	0.125g (12,500IU)
19	Beta-D-glucose	5	
20	Lactoperoxidase (100,000 IU/g)	0.0001 g (10 IU)	
21	Potassium thiocyanate	0.01	
22	Color	0.5	0.5
23	Flavor	0.5	0.5

24 In the above formulations, Composition 1A corresponds
 25 to the instant invention while Composition 1B simulates the
 26 prior art. The compositions were prepared by blending the dry
 27 ingredients into the liquid ingredients, with agitation, until a
 28 smooth admixture was obtained.

29 Ten individuals rinsed their mouths for five minutes
 30 with an aqueous sugar solution containing 25 wt. % sucrose and
 31 25 wt % glucose. The ten individuals were divided into five

1 groups, with two persons to a group. Saliva samples were
2 separately collected from the ten individuals in accordance with
3 the following time sequence: group 1, immediately after rinsing;
4 group 2, 60 minutes after rinsing; group 3, 120 minutes after
5 rinsing; group 4, 180 minutes after rinsing; and group 5, 240
6 minutes after rinsing. One individual in each group was desig-
7 nated "A" and the other individual in each group was designated
8 "B".

9 Ten bacterial specimens were prepared by pouring
10 10 ml of Brain-Heart Infusion agar containing 10,000 colony
11 units of streptococcus mutans (strain C67-1) per ml into each
12 of 10 Petri dishes, as needed, with one dish in each set of two
13 dishes being designated "A" and the dish being designated "B".
14

15 Promptly following the collection of saliva from
16 individuals "A" and "B" in each time period, 5 ml of saliva from
17 individual "A" and 1.0 ml of Toothpaste Composition 1A were
18 added with stirring to Petri dish "A" and 5 ml of saliva from
19 individual "B" and 1.0 ml of Toothpaste Composition 1B were
20 added with stirring to Petri dish "B". The resulting admixtures
21 were incubated in an oven at 35° C for 10 minutes. Upon com-
22 pletion of the incubation period, the bacterial specimen ad-
23 mixture were removed from the oven and microscopically evaluated
24 for bacterial inhibition as determined by visible colony count.
25 The results of this comparative study are set forth in Table
26 IV.
27
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1 TABLE IV
2

3 Group	Time, minutes after rinse when ingredients added to bacterial broth	Percent Bacterial Inhibition		
		4 Saliva "A" Composition 1A	5 Saliva "B" Composition 1B	6
1 1	immediately	99	99	
2 2	60	99	78	
3 3	120	99	59	
4 4	180	99	42	
5 5	240	99	38	

9 Since glucose concentration in the oral cavity decreases with increasing time lapse following a sugar rinse,
 10 the results set forth in Table IV show that the di-enzymatic
 11 compositions of this invention maintain significant anti-
 12 bacterial effectiveness in an oral environment of declining
 13 glucose concentration whereas the antibacterial effectiveness
 14 of enzymatic compositions of the prior art containing glucose
 15 oxidase as the essential active ingredient decrease with
 16 declining glucose concentration.
 17
 18

EXAMPLE 2

The following examples show varying ingredients and concentration levels which can be used in the preparation of di-enzymatic toothpaste compositions.

2A

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	50
Calcium pyrophosphate	40
Sodium bicarbonate	5
Water	1.5
Super-Pro	2
Glucose oxidase (100,000 IU/g)	0.1 (10,000 IU)
Beta-D-glucose	0.5
Lactoperoxidase (100,000 IU/g)	0.002 (200 IU)
Sodium thiocyanate	0.04
Color	0.5
Flavor	0.5

2B

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	47
Calcium pyrophosphate	40
Titanium dioxide	5
Water	3
Sodium lauryl sulphate	2
Glucose oxidase (100,000 IU/g)	0.4 (40,000 IU)
Beta-D-glucose	2
Lactoperoxidase (100,000 IU/g)	0.008 (800 IU)
Potassium thiocyanate	0.002
Color	0.5
Flavor	0.5

2C

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	50
Calcium pyrophosphate	40
Sodium bicarbonate	5
Water	1.5
D-amino acid oxidase (100,000 IU/g)	0.1 (10,000 IU)
D-alanine	0.5
Lactoperoxidase (100,000 IU/g)	0.002 (200 IU)
Sodium thiocyanate	0.04
Color	0.5
Flavor	0.5

2D

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	46
Titanium dioxide	2
Silcron G-910	40
Water	2
Maypon	2
Glucose oxidase (100,000 IU/g)	0.05 (5,000 IU)
Beta-D-glucose	1
Lactoperoxidase (100,000 IU/g)	0.01 (1,000 IU)
Potassium thiocyanate	0.005
Color	0.5
Flavor	0.5

2E

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	46
Titanium dioxide	2
Silcron G-910	40
Water	2
Maypon	2
D-glutamate oxidase (100,000 IU/g)	0.05 (5,000 IU)
D-glutamate	1
Lactoperoxidase (100,000 IU/g)	0.01 (1,000 IU)
Sodium thiocyanate	0.08
Color	0.5
Flavor	0.5

2F

<u>Composition</u>	<u>weight, grams</u>
Propylene glycol	48
Dicalcium phosphate	45
Water	3.5
Super-Pro	2
Glucose oxidase (100,000 IU/g)	0.0008 (80 IU)
Beta-D-glucose	0.5
Lactoperoxidase (100,000 IU/g)	0.005 (500 IU)
Sodium thiocyanate	0.01
Color	0.5
Flavor	0.5

2G

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	50
Calcium pyrophosphate	40
Dicalcium phosphate	5
Water	2
Glucose oxidase (100,000 IU/g)	0.05 (5,000 IU)
Beta-D-glucose	1
Choline oxidase (100,000 IU/g)	0.02 (2,000 IU)
Choline	1
Lactoperoxidase (100,000) IU/g)	0.008 (800 IU)
Potassium thiocyanate	0.009
Color	0.5
Flavor	0.5

2H

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	42
Dicalcium phosphate	6
Titanium dioxide	2
Silcron G-910	38
Water	5
Glucose oxidase (100,000 IU/g)	0.4 (40,000 IU)
Beta-D-glucose	6
Lactoperoxidase (100,000 IU/g)	0.001 (100 IU)
Sodium thiocyanate	0.01
Color	0.5
Flavor	0.5

2I

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	42
Dicalcium phosphate	6
Titanium dioxide	2
Silcron G-910	38
Water	5
Glucose oxidase (100,000 IU/g)	0.02 (2,000 IU)
Beta-D-glucose	1
Lactoperoxidase (100,000 IU/g)	0.001 (100 IU)
Sodium thiocyanate	0.01
Color	0.5
Flavor	0.5

2JCompositionweight, grams

3	Glycerine (99%)	50
4	Titanium dioxide	2
5	Silcron G-910	40
6	Water	2
7	Super-Pro	2
8	Glucose oxidase (100,000 IU/g)	0.02 (2,000 IU)
9	Beta-D-glucose	2
10	Lactoperoxidase (100,000 IU/g)	0.01 (1,000 IU)
11	Sodium thiocyanate	0.01
12	Color	0.5
13	Flavor	0.5

2KCompositionweight, grams

12	Propylene glycol	44
13	Sodium bicarbonate	5
14	Silcron G-910	40
15	Water	6.4
16	Super-Pro	2
17	Glucose oxidase (100,000 IU/g)	0.025 (2,500 IU)
18	Beta-D-glucose	1.5
19	Lactoperoxidase (100,000 IU/g)	0.006 (600 IU)
20	Potassium thiocyanate	0.005
21	Color	0.5
22	Flavor	0.5
23	N-acetyl glucosamine	0.15

2LCompositionweight, grams

21	Propylene glycol	48
22	Sodium bicarbonate	5
23	Silcron G-910	40
24	Water	2.4
25	Super-Pro	2
26	Glucose oxidase (100,000 IU/g)	0.025 (2,500 IU)
27	Beta-D-glucose	1.5
28	Lactoperoxidase (100,000 IU/g)	0.0005 (50 IU)
29	Potassium thiocyanate	0.005
30	Color	0.5
31	Flavor	0.5
32	Glucosamine	0.1

2M

<u>Composition</u>	<u>weight, grams</u>
Glycerine (99%)	47
Sodium bicarbonate	5
Silcron G-910	40
Water	3.5
Super-Pro	2
Glucose oxidase (100,000 IU/g)	0.04 (4,000 IU)
Beta-D-glucose	1.5
Lactoperoxidase (100,000 IU/g)	0.012 (1,200 IU)
Sodium thiocyanate	0.05
Color	0.5
Flavor	0.5
Glucosamine	0.012
N-acetyl glucosamine	0.01

In view of the foregoing description and examples, it will become apparent to those of ordinary skill in the art that equivalent modifications thereof may be made without departing from the spirit and scope of this invention.

1 The embodiments of the invention in which an exclusive
2 property or privilege is claimed are defined as follows:

3 1. A di-enzymatic dentifrice containing from about 0.015 to
4 about 0.6 millimole of oxidizable substrate and from about 0.5
5 to about 500 International Units of an oxidoreductase enzyme
6 specific to such substrate for producing hydrogen peroxide upon
7 oral application of said dentifrice and further containing from
8 about 0.0001 to about 0.01 millimole of a thiocyanate salt and
9 from about 0.05 to about 20 International Units of lactoper-
10 oxidase for interacting with hydrogen peroxide to produce a
11 hypothiocyanate bacterial inhibitor, wherein each of the afore-
12 said quantities is based upon one gram of dentifrice; and
13 limiting any water present in the dentifrice to an amount not
14 more than about 10 wt. % based on the dentifrice weight to
15 stabilize the dentifrice against the production of hydrogen per-
16 oxide prior to the oral application of the dentifrice.

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19 2. The dentifrice of claim 1 wherein the oxidizable
20 substrate is Beta-D-glucose and the oxidoreductase enzyme is
21 glucose oxidase.

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24 3. The dentifrice of claim 1 wherein the oxidizable
25 substrate is D-galactose and the oxidoreductase enzyme is
26 galactose oxidase.

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1 4. The dentrifice of claim 1 wherein the oxidizable
2 substrate is urate and the oxidoreductase enzyme is urate
3 oxidase.

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6 5. The dentrifice of claim 1 wherein the oxidizable
7 substrate is choline and the oxidoreductase enzyme is choline
8 oxidase.

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11 6. The dentrifice of claim 1 wherein the oxidizable
12 substrate is a D-amino acid selected from the group consisting
13 of D isomers of proline, methionine, isoleucine, alanine,
14 valine and phenylalanine and the oxidoreductase enzyme is
15 D-amino acid oxidase.

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18 7. The dentrifice of claim 1 wherein the substrate is
19 D-glutamate and the oxidoreductase enzyme is D-glutamate
20 oxidase.

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26 8. The dentrifice of claim 1 wherein the oxidizable
27 substrate is glycine and the oxidoreductase enzyme is glycine
28 oxidase.

1 9. The dentrifice of claim 1 wherein the thiocyanate
2 salt is a member selected from the group consisting of sodium
3 thiocyanate, potassium thiocyanate, ammonium thiocyanate and
4 mixture thereof.

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7 10. The dentrifice of claim 1 which also contains an
8 aminoglucose selected from the group consisting of glucosamine,
9 N-acetyl glucosamine and mixture thereof in an amount from
10 about 0.001 to about 0.002 millimole per gram of dentrifice.

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14 11. The dentrifice of claim 1 wherein the oxidizable
15 substrate is present in an amount from about 0.025 to about
16 0.1 millimole per gram of dentrifice.

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19 12. The dentrifice of claim 1 wherein the oxidoreductase
20 enzyme is present in an amount from about 10 to about 40
21 International Units per gram of dentrifice.

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24 13. The dentrifice of claim 1 wherein the thiocyanate
25 salt is present in an amount from about 0.001 to about 0.006
26 millimole per gram of dentrifice.

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1 14. The dentrifrice of claim 1 wherein lactoperoxidase
2 is present in an amount from about 0.1 to about 1.0 Internation-
3 al Units per gram of dentrifice.

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6 15. The dentrifrice of claim 9 wherein the aminoglucose
7 is present in amount from about 0.0003 to about 0.001 millimole
8 per gram of dentrifice.

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11 16. The dentrifrice of claim 1 wherein the oxidizable
12 substrate is glucose which is present in an amount from about
13 0.025 to about 0.1 millimole per gram of dentrifice, the
14 oxidoreductase enzyme is glucose oxidase which is present
15 in amount from about 10 to about 40 International Units per
16 gram of dentrifice, the thiocyanate salt is present in an
17 amount from about 0.001 to about 0.006 millimole per gram
18 of dentrifice, and lactoperoxidase is present in an amount
19 from about 0.1 to about 1.0 International Unit per gram of
20 dentrifice.

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23 17. The dentrifrice of claim 16 which also contains an
24 aminoglucose selected from the group consisting of glucosamine,
25 N-acetyl glucosamine and mixture thereof in an amount from
26 about 0.0003 to about 0.001 millimole per gram of dentrifice.

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SUBSTITUTE

REEMPLACEMENT

SECTION is not Present

Cette Section est Absente